इंटरनेट मानक

Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS/ISO 14852 (1999): Determination of ultimate aerobic diodegradability of plastic materials in and aqueous medium - Method by analysis of evolved carbon dioxide [PCD 12: Plastics]

"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

Act Number 22 of 2005
The Indian Parliament
RIGHT TO INFORMATION

Made Available By
Public.Resource.Org

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

IS/ISO 14852 : 1999

भारतीय मानक

जलीय माध्यम में प्लास्टिक सामग्री की अंतिम (अल्टीमेट) ऑक्सी जैव विघट्नीयता ज्ञात करना — उत्पन्न कार्बन डाइऑक्साइड के विश्लेषण द्वारा पद्धति

Indian Standard

DETERMINATION OF THE ULTIMATE AEROBIC BIODEGRADABILITY OF PLASTIC MATERIALS IN AN AQUEOUS MEDIUM — METHOD BY ANALYSIS OF EVOLVED CARBON DIOXIDE

ICS 83.010.01

© BIS 2009

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

January 2009

Price Group 8

Plastics Sectional Committee, PCD 12

NATIONAL FOREWORD

This Indian Standard which is identical with ISO 14852 : 1999 'Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Plastics Sectional Committee and approval of the Petroleum, Coal and Related Products Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker in the International Standard while in Indian Standard, the current practice is to use a point (.) as the decimal marker.

The technical committee responsible for the preparation of this standard has reviewed the provisions of the following International Standards and has decided that they are acceptable for use in conjunction with this standard:

International Standard	*Title*
ISO 8245 : 1999	Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)
ISO 9439 : 1999	Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evaluation test
ISO 10634 : 1995	Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium
ISO/TR 15462 : 1997	Water quality — Selection of tests for biodegradability

Technical Corrigendum 1 to the above International Standard has been given at the end of this standard.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

DETERMINATION OF THE ULTIMATE AEROBIC BIODEGRADABILITY OF PLASTIC MATERIALS IN AN AQUEOUS MEDIUM — METHOD BY ANALYSIS OF EVOLVED CARBON DIOXIDE

WARNING — Sewage, activated sludge, soil and compost may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This International Standard specifies a method, by measuring the amount of carbon dioxide evolved, for the determination of the degree of aerobic biodegradability of plastic materials, including those containing formulation additives. The test material is exposed in a synthetic medium under laboratory conditions to an inoculum from activated sludge, compost or soil.

If an unadapted activated sludge is used as the inoculum, the test simulates the biodegradation processes which occur in a natural aqueous environment; if a mixed or pre-exposed inoculum is used, the method can be used to investigate the potential biodegradability of a test material.

The conditions used in this International Standard do not necessarily correspond to the optimum conditions allowing maximum biodegradation to occur, but the standard is designed to determine the potential biodegradability of plastic materials or give an indication of their biodegradability in natural environments.

The method enables the assessment of the biodegradability to be improved by calculating a carbon balance (optional, see annex C).

The method applies to the following materials:

- Natural and/or synthetic polymers, copolymers or mixtures thereof.
- Plastic materials which contain additives such as plasticizers, colorants or other compounds.
- Water-soluble polymers.
- Materials which, under the test conditions, do not inhibit the microorganisms present in the inoculum. Inhibitory effects can be determined using an inhibition control or by another appropriate method (see e.g. ISO 8192[2]). If the test material is inhibitory to the inoculum, a lower test concentration, another inoculum or a pre-exposed inoculum can be used.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 8245:1999, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).*

ISO 9439 —[1], *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test.*

ISO 10634:1995, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.*

ISO/TR 15462:1997, *Water quality — Selection of tests for biodegradability.*

3 Definitions

For the purposes of this International Standard, the following definitions apply:

3.1
ultimate aerobic biodegradation
the breakdown of an organic compound by microorganisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) plus new biomass

3.2
activated sludge
biomass produced in the aerobic treatment of waste water by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

3.3
concentration of suspended solids in an activated sludge
the amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

3.4
dissolved inorganic carbon
DIC
that part of the inorganic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter

3.5
theoretical amount of evolved carbon dioxide
$ThCO_2$
the maximum theoretical amount of carbon dioxide evolved after completely oxidizing a chemical compound, calculated from the molecular formula and expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound

3.6
total organic carbon
TOC
all the carbon present in organic matter which is dissolved or suspended in water

3.7
dissolved organic carbon
DOC
that part of the organic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter

---

[1] To be published. (Revision of ISO 9439:1990)

3.8

lag phase

the time, measured in days, from the start of a test until adaptation and/or selection of the degrading microorganisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation

3.9

maximum level of biodegradation

the degree of biodegradation, measured in per cent, of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

3.10

biodegradation phase

the time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

3.11

plateau phase

the time, measured in days, from the end of the biodegradation phase until the end of a test

3.12

pre-exposure

the pre-incubation of an inoculum in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the inoculum to biodegrade the test material by adaptation and/or selection of the microorganisms

3.13

pre-conditioning

the pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the test by acclimatization of the microorganisms to the test conditions

4 Principle

The biodegradability of a plastic material is determined using aerobic microorganisms in an aqueous system. The test mixture contains an inorganic medium, the organic test material (the sole source of carbon and energy) with a concentration between 100 mg/l and 2 000 mg/l of organic carbon, and activated sludge or a suspension of active soil or compost as the inoculum. The mixture is agitated in test flasks and aerated with carbon-dioxide-free air over a period of time depending on the biodegradation kinetics, but not exceeding 6 months. The carbon dioxide evolved during the microbial degradation is determined by a suitable analytical method, examples of which are given in annexes A and B.

The level of biodegradation is determined by comparing the amount of carbon dioxide evolved with the theoretical amount ($ThCO_2$) and expressed in per cent. The test result is the maximum level of biodegradation, determined from the plateau phase of the biodegradation curve. Optionally, a carbon balance may be calculated to give additional information on the biodegradation (see annex C).

Unlike ISO 9439, which is used for a variety of organic compounds, this International Standard is specially designed for the determination of the biodegradability of plastic materials. The special requirements necessary affect the choice of the inoculum and the test medium, and there is the possibility of improving the evaluation of the biodegradability by calculating a carbon balance.

5 Test environment

Incubation shall take place in the dark or in diffuse light in an enclosure which is free from vapours inhibitory to microorganisms and which is maintained at a constant temperature, preferably between 20 °C and 25 °C, to an accuracy of ± 1 °C, or at any other appropriate temperature depending on the inoculum used and the environment to be assessed.

NOTE With a compost inoculum, higher temperatures may be appropriate.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Distilled or deionized water, free of toxic substances (copper in particular) and containing less than 2 mg/l of DOC.

6.2 Test medium.

Depending on the purpose of the test, different test media may be used. For example, if simulating a natural environment use the standard test medium (6.2.1). If a test material is used at higher concentrations, use the optimized test medium (6.2.2) with higher buffering capacity and nutrient concentrations.

6.2.1 Standard test medium

6.2.1.1 Solution A

Dissolve

anhydrous potassium dihydrogen phosphate (KH_2PO_4)	8,5 g
anhydrous dipotassium hydrogen phosphate (K_2HPO_4)	21,75 g
disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	33,4 g
ammonium chloride (NH_4Cl)	0,5 g

in water (6.1) and make up to 1 000 ml.

NOTE The correct composition of the solution can be checked by measuring the pH, which should be 7,4.

6.2.1.2 Solution B

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (6.1) and make up to 1 000 ml.

6.2.1.3 Solution C

Dissolve 36,4 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (6.1) and make up to 1 000 ml.

6.2.1.4 Solution D

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1) and make up to 1 000 ml.

Prepare this solution freshly before use to avoid precipitation, or add a drop of concentrated hydrochloric acid (HCl) or a drop of 0,4 g/l aqueous solution of ethylenediaminetetraacetic acid (EDTA).

6.2.1.5 Preparation

To prepare 1 litre of test medium, add, to about 500 ml of water (6.1),

- 10 ml of solution A;
- 1 ml of each of solutions B to D.

Make up to 1 000 ml with water (6.1).

6.2.2 Optimized test medium

This optimized medium is highly buffered and contains more inorganic nutrients. This is necessary to keep the pH constant in the system during the test, even at high concentrations of the test material. The medium contains about 2 400 mg/l of phosphorus and 50 mg/l of nitrogen and is therefore suitable for concentrations in the test material of up to 2 000 mg/l of organic carbon. If higher or lower test-material concentrations are used, increase or decrease respectively the nitrogen content to keep the C:N ratio at about 40:1.

6.2.2.1 Solution A

Dissolve

anhydrous potassium dihydrogen phosphate (KH_2PO_4)	37,5 g
disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	87,3 g
ammonium chloride (NH_4Cl)	2,0 g

in water (6.1) and make up to 1 000 ml.

6.2.2.2 Solution B

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (6.1) and make up to 1 000 ml.

6.2.2.3 Solution C

Dissolve 36,4 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (6.1) and make up to 1 000 ml.

6.2.2.4 Solution D

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1) and make up to 1 000 ml (see second paragraph of 6.2.1.4).

6.2.2.5 Solution E (trace-element solution, optional)

Dissolve in 10 ml of aqueous HCl solution (25 %, 7,7 mol/l), in the following sequence:

70 mg of $ZnCl_2$, 100 mg of $MnCl_2 \cdot 4H_2O$, 6 mg of H_3BO_3, 190 mg of $CoCl_2 \cdot 6H_2O$, 3 mg of $CuCl_2 \cdot 2H_2O$, 240 mg of $NiCl_2 \cdot 6H_2O$, 36 mg of $Na_2MoO_4 \cdot 2H_2O$, 33 mg of $Na_2WO_4 \cdot 2H_2O$ and 26 mg of $Na_2SeO_3 \cdot 5H_2O$

and make up to 1 000 ml with water (6.1).

6.2.2.6 Solution F (vitamin solution, optional)

Dissolve in 100 ml of water (6.1) 0,6 mg of biotine, 2,0 mg of niacinamide, 2,0 mg of *p*-aminobenzoate, 1,0 mg of panthotenic acid, 10,0 mg of pyridoxal hydrochloride, 5,0 mg of cyanocobalamine, 2,0 mg of folic acid, 5,0 mg of riboflavin, 5,0 mg of DL-thioctic acid and 1,0 mg of thiamine dichloride or use a solution of 15 mg of yeast extract in 100 ml of water (6.1). Filter the solution for sterilization using membrane filters (see 7.6).

NOTE Solutions E and F are optional and are not required if a sufficient concentration of the inoculum is used, e.g. activated sludge, soil or compost. It is recommended that 1 ml portions be prepared and kept refrigerated until use.

6.2.2.7 Preparation

To prepare 1 litre of test medium, add, to about 800 ml of water (6.1),

- 100 ml of solution A;
- 1 ml of each of solutions B to D and, optionally, E and F.

Make up to 1 000 ml with water (6.1) and measure the pH.

NOTE The correct composition of the test medium can be checked by measuring the pH, which should be 7,0 ± 0,2.

6.3 Pyrophosphate solution.

Dissolve 2,66 g of sodium pyrophosphate ($Na_4P_2O_7$) in water (6.1) and make up to 1 000 ml.

7 Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter.

Required is usual laboratory equipment, plus the following:

7.1 **Test flasks:** glass vessels (e.g. bottles or conical flasks) designed to allow gas purging and shaking or stirring, and fitted with tubing impermeable to CO_2. The vessels shall be located in a constant-temperature room or in a thermostatted apparatus (e.g. water-bath).

7.2 **CO_2-free-air production system,** capable of supplying CO_2-free air at a flow rate between 50 ml/min and 100 ml/min to each test flask, held constant to within ± 10 % (see example of system, including test vessels, in annex A).

7.3 **Analytical instrument for determining carbon dioxide,** consisting of any suitable apparatus with sufficient accuracy, e.g. a CO_2 or DIC analyser or apparatus for titrimetric determination after complete absorption in a basic solution (see examples in annex B). Note that, if an analyser with an IR detector, for instance, is used, CO_2-free air is not necessary.

7.4 **Analytical equipment for measuring total organic carbon (TOC) and dissolved organic carbon (DOC)** (see ISO 8245).

7.5 **Analytical balance** (usual laboratory equipment).

7.6 **Centrifuge,** or **filtration device** with membrane filters (0,45 µm pore size) which neither adsorb nor release organic carbon significantly.

7.7 **pH meter** (usual laboratory equipment).

7.8 **Magnetic stirrer or shaking device** (usual laboratory equipment).

8 Procedure

8.1 Test material

The test material shall be of known mass and contain sufficient carbon to yield CO_2 in a quantity that can be adequately measured by the analytical system used. Calculate the TOC from the chemical formula or determine it by a suitable analytical technique (e.g. elemental analysis or measurement in accordance with ISO 8245) and calculate the $ThCO_2$. Use a concentration of test material such that the TOC content is at least 100 mg/l. The maximum amount of test material is limited by the oxygen supply to the test system and the test medium used. When using the optimized test medium (6.2.2) the test-material concentration shall be such that the TOC does not exceed about 2 000 mg/l, i.e. a C:N ratio of about 40:1. If higher concentrations are to be tested, increase the nitrogen amount in the test medium.

NOTE The test material should preferably be used in powder form, but it may also be introduced as films, pieces, fragments or shaped articles. The form and shape of the test material may influence its biodegradability. Similar shapes should preferably be used if different kinds of plastic material are to be compared. If the test material is used in the form of a powder, particles of known, narrow size distribution should be used. A particle-size distribution with the maximum at 250 µm diameter is recommended. Also, the size of the test equipment used may depend on the form of the test material. It should be ascertained that no substantial mechanical aberrations occur due to the test conditions, for example due to the type of stirring mechanism used. Processing of the test material (e.g. the use of powder in the case of composites) should not influence significantly the degradation behaviour of the material. Optionally, record the hydrogen, oxygen, nitrogen, phosphorus and sulfur contents and the molecular mass of a polymeric test material, using for example liquid exclusion chromatography (see e.g. ASTM D 3536-91[1] or any other applicable standard method). Preferably, plastic materials without additives such as plasticizers should be tested. When the material does contain such additives, information on their biodegradability will be needed to assess the biodegradability of the polymeric material itself.

For details on how to handle poorly water-soluble compounds, see ISO 10634.

8.2 Reference material

Use aniline and/or a well defined biodegradable polymer (for example microcrystalline cellulose powder, ashless cellulose filters or poly-β-hydroxybutyrate) as a reference material. If possible, the TOC, form and size should be comparable to that of the test material.

As a negative control, a non-biodegradable polymer (e.g. polyethylene) in the same form as the test material can optionally be used.

8.3 Preparation of the inoculum

Activated sludge from a sewage-treatment plant treating predominantly domestic sewage is a suitable source of the inoculum. It is obtained from an active aerobic environment and is available over a wide geographical area in which a broad range of plastic materials has to be tested. Alternatively, soil and/or compost suspensions can be used for inoculation, as with some plastic materials the activity of fungi is important for biodegradation. When biodegradation in a specific waste-treatment system is to be determined, collect the inoculum from that environment.

The inoculum can be prepared from the sources described in 8.3.1 and 8.3.2, or from a mixture of these sources in order to obtain a varied and concentrated microbial flora with sufficient biodegradation activity. If the endogenous respiration of the inoculum is too high, stabilize the inoculum by aeration before use. Harmonize the test temperature with the inoculum used (see note to clause 5).

NOTE It may be useful to determine the colony-forming units (cfu) of the inoculum used. The test mixture should preferably contain about 10^{-6} cfu/ml.

8.3.1 Inoculum from wastewater-treatment plants

Take a sample of activated sludge collected from a well-operated sewage-treatment plant or a laboratory plant handling predominantly domestic sewage. Mix well, keep the sample under aerobic conditions and use preferably on the day of collection (at least within 72 h).

Before use, determine the concentration of suspended solids (use e.g. ISO 11923[3]). If necessary, concentrate the sludge by settling so that the volume of sludge added to the test assay is minimal. Add a suitable volume to obtain suspended solids in the range 30 mg/l to 1 000 mg/l in the final mixture.

NOTE 1 When biodegradation processes in a natural environment are to be simulated or when a carbon balance determination (see annex C) is to be carried out, an inoculum concentration of 30 mg/l suspended solids is recommended. As solid matter can interfere with the carbon balance determination, the following procedure for preparing the inoculum is recommended. Take 500 ml of the activated sludge and homogenize for 2 min at medium speed in a blender or in a suitable high-speed mixer. Allow to settle until the supernatant liquid contains no significant amounts of suspended matter, but in any case for at least 30 min. Decant a sufficient volume of the supernatant liquid and add it to the test flasks to obtain a concentration of 1 % (V/V) to 5 % (V/V) in the test medium. Avoid carrying over sludge particles.

NOTE 2 An inoculum may be pre-conditioned, but normally no pre-exposed inoculum should be used, especially in the case of standard tests simulating biodegradation behaviour in natural environments. Depending on the purpose of the test, a pre-exposed inoculum may also be used, provided this is clearly stated in the test report (e.g. per cent biodegradation $= x$ %, using pre-exposed inocula) and the method of pre-exposure detailed in the test report. Pre-exposed inocula can be obtained from suitable laboratory biodegradation tests (see ISO/TR 15462) conducted under a variety of conditions or from samples collected from locations where relevant environmental conditions exist (e.g. contaminated areas or industrial treatment plants).

8.3.2 Inoculum from soil and/or compost

Suspend 10 g of non-sterile, fertile soil or compost from a composting plant treating predominantly organic waste in 100 ml of the test medium (6.2.1 or 6.2.2) or in a pyrophosphate solution (6.3) which is commonly used in soil microbiology. Allow to settle for about 30 min. Decant and filter the supernatant liquid through a coarse porous filter and add the inoculum to the test flasks to obtain a concentration of 1 % (V/V) to 5 % (V/V) in the test medium. Higher amounts of inoculum can be used if necessary, but this may cause problems in establishing carbon balances. The use of compost can increase the number of fungi in the test flasks and improve the biodegradation of plastic materials. In this case, indicate the state of the compost used in the test report (e.g. mature compost, compost from the hot phase at about 50 °C).

8.4 Test

Provide a number of flasks, so that the test includes at least the following:

a) Two test flasks for the test material (symbol F_T).

b) Two flasks for the blank (symbol F_B).

c) One flask for checking the inoculum activity using a reference material (symbol F_C).

And, if required:

d) One flask for checking for possible abiotic degradation or non-biological change in the test material such as by hydrolysis (symbol F_S). The test solution in F_S shall be sterilized, for example by autoclaving or by the addition of a suitable inorganic toxic compound to prevent microbial activity. Use, for example, 5 ml/l of a solution containing 10 g/l of mercury(II) chloride ($HgCl_2$). Add the same amount of the toxic substance during the test if required.

e) One flask as a negative control (symbol F_N) using a non-biodegradable polymeric substance (e.g. polyethylene) in the same form as the test material.

f) One flask for checking the possible inhibiting effect of the test material on microbial activity (symbol F_I). Take care that the ratio of carbon in the test and reference material to nitrogen in the medium is at least about C:N = 40:1. Add nitrogen if required.

Add appropriate amounts of the test medium (6.2) and the inoculum (see 8.3) to the test flasks as indicated in table 1.

Table 1 — Final distribution of test and reference materials

Flask	Test material	Reference material	Inoculum
F_T Test	+	–	+
F_T Test	+	–	+
F_B Blank	–	–	+
F_B Blank	–	–	+
F_C Inoculum check	–	+	+
F_S Abiotic degradation check (optional)	+	–	–
F_I Inhibition control (optional)	+	+	+
F_N Negative control (optional)	–	+	+

Connect the flasks to the CO_2-free-air production system (see annex A). Incubate at the desired test temperature (see clause 5) and aerate the flasks for 24 h to purge carbon dioxide from the system. At higher temperatures, prevent any ingress or loss of liquid by means of suitable equipment. Agitate throughout the test with a magnetic stirrer or shaker. If excessive foaming is observed, replace the air purge by overhead aeration with stirring. After the pre-aeration period, connect the air exit of each flask to the carbon dioxide trapping or measuring system.

If a carbon balance is to be run (see annex C), remove a known sufficient volume of the inoculated test medium from each flask or from additional separate flasks for DOC and biomass determination at the beginning and the end of the incubation period. Consider the removed volume when adjusting the final volume or when calculating the test results.

Add the test material (8.1), the reference material and the material for the negative control (8.2) to the respective flasks as indicated in table 1 and start the test by bubbling CO_2-free air through the flasks to ensure a sufficient quantity of oxygen thoughout the test. A rate of 50 ml/min to 100 ml/min is usually suitable.

Measure at regular intervals, depending on the carbon dioxide evolution rate, the amount of carbon dioxide evolved from each bottle, using a suitable and sufficiently accurate method (see annex B).

When a constant level of carbon dioxide release is attained (plateau phase reached) and no further biodegradation is expected, the test is considered to be completed. The maximum test period is 6 months. In the case of long test durations, special attention must be paid to the technical system (e.g. tightness of the test vessels and connections, ensuring no carbon dioxide enters and ensuring there are no leakages).

On the last day of the test, measure the pH, acidify all the bottles with 1 ml of concentrated hydrochloric acid in order to decompose the carbonates and bicarbonates, and purge to remove the carbon dioxide. Continue aeration for 24 h and measure the amount of carbon dioxide evolved in each of the series of flasks (F_T, F_B, F_C,...).

9 Calculation and expression of results

9.1 Calculation

9.1.1 Theoretical amount of carbon dioxide evolved by the test material

Calculate the theoretical amount of carbon dioxide ($ThCO_2$) evolved, expressed in milligrams, using equation (1).

$$\mathrm{ThCO_2} = m \times X_\mathrm{C} \times \frac{44}{12} \qquad \ldots (1)$$

where

m is the mass of test material introduced into the test system, in milligrams;

X_C is the carbon content of the test material, determined from the chemical formula or calculated from an elemental anlysis and expressed as a mass fraction;

44 and 12 are the molecular mass of carbon dioxide and the atomic mass of carbon, respectively.

Calculate in the same way the theoretical amount of carbon dioxide evolved by the reference material and the mixture of test and reference material in flask F_I.

9.1.2 Percentage biodegradation from CO_2 evolution

Calculate the percentage biodegradation D_t for the test flasks F_T from the amount of carbon dioxide evolved for each measurement interval using equation (2)

$$D_t = \frac{\sum(\mathrm{CO_2})_\mathrm{T} - \sum(\mathrm{CO_2})_\mathrm{B}}{\mathrm{ThCO_2}} \times 100 \qquad \ldots (2)$$

where

$\Sigma(\mathrm{CO_2})_\mathrm{T}$ is the amount of carbon dioxide evolved in flask F_T between the start of the test and time t, expressed in milligrams;

$\Sigma(\mathrm{CO_2})_\mathrm{B}$ is the amount of carbon dioxide evolved in the blank flask F_B between the start of the test and time t, expressed in milligrams;

$\mathrm{ThCO_2}$ is the theoretical amount of carbon dioxide evolved by the test material, expressed in milligrams.

If possible, calculate the average for the duplicate flasks. In the same way, calculate the percentage biodegradation of the reference material in the inoculum check flask F_C and, if included, the percentage biodegradation of the mixture of test and reference material in the inhibition control F_I, the test material in the abiotic degradation control F_S and the negative control F_N.

If a carbon balance is to be run, calculate the degree of biodegradation of the test material from the amount of carbon dioxide evolved and the carbon content of the biomass formed during the test (see annex C).

9.2 Expression and interpretation of results

Compile a table of carbon dioxide released and the percentage biodegradation for each measurement interval and each test flask. For each vessel, plot a curve of the carbon dioxide evolved and a curve of the percentage biodegradation as a function of time. If comparable results are obtained for the duplicate flasks, a mean curve may be plotted.

The maximum level of biodegradation determined as the mean value of the plateau phase of the biodegradation curve or the highest value, e.g. when the curve decreases or, further on, slowly increases in the plateau phase, characterizes the degree of biodegradation of the test material. If a carbon balance has been determined, the result of this determination characterizes the total degree of biodegradation.

The wettability and the shape of the test material may influence the result obtained, and hence the test procedure may be limited to comparing plastic materials of similar chemical structure.

Information on the toxicity of the test material may be useful in the interpretation of test results showing a low biodegradability.

10 Validity of results

The test is considered valid if

a) the degree of biodegradation of the reference material (inoculum check F_C) is > 60 % at the end of the test;

b) the amount of carbon dioxide which has evolved from the blank F_B at the end of the test does not exceed an upper limiting value obtained by experience (this value depends on the amount of inoculum and is, for example, in the case of 30 mg/l dry matter, about 90 mg/l as interlaboratory tests have shown).

If in flask F_I (inhibition check, if included) the percentage biodegradation is < 25 % and no significant degradation of the test material is observed, it can be assumed that the test material is inhibitory.

If in flask F_S (abiotic degradation check, if included) a significant amount (> 10 %) of evolved carbon dioxide is observed, abiotic degradation processes may have taken place.

If flask F_N (negative control) was included, no significant amount of evolved carbon dioxide shall be observed.

If these criteria are not fulfilled, repeat the test using another pre-conditioned or pre-exposed inoculum.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) all information necessary to identify the test and reference materials, including their TOC, $ThCO_2$, chemical composition and formula (if known), shape, form and amount/concentration in the samples tested;

c) the main test parameters, including test volume, test medium used, incubation temperature and final pH;

d) the source and amount of the inoculum used, including details of any pre-exposure and the state of the compost used;

e) the analytical techniques used, including methods of carbon dioxide detection and TOC, DOC and biomass determination;

f) all the test results obtained for the test and reference materials (in tabular and graphical form), including the measured accumulated carbon dioxide, the percentage biodegradation values and the respective curves of these parameters against time;

g) the duration of the lag phase, biodegradation phase and maximum level of degradation, as well as the total test duration;

and, optionally, if run or determined:

h) the results of the abiotic degradation check F_S, the inhibition control F_I and the negative control F_N;

i) the results of the carbon balance determination, including for example

  1) the amount of carbon in the test material oxidized to carbon dioxide,

  2) the increase in DOC in the test medium during the incubation period due to water-soluble substances,

  3) the increase in organic carbon in the biomass during the test,

  4) the carbon content of the residual polymers at the end of the test,

  5) the sum of all the carbon measured, expressed as a percentage of the carbon introduced as the test material;

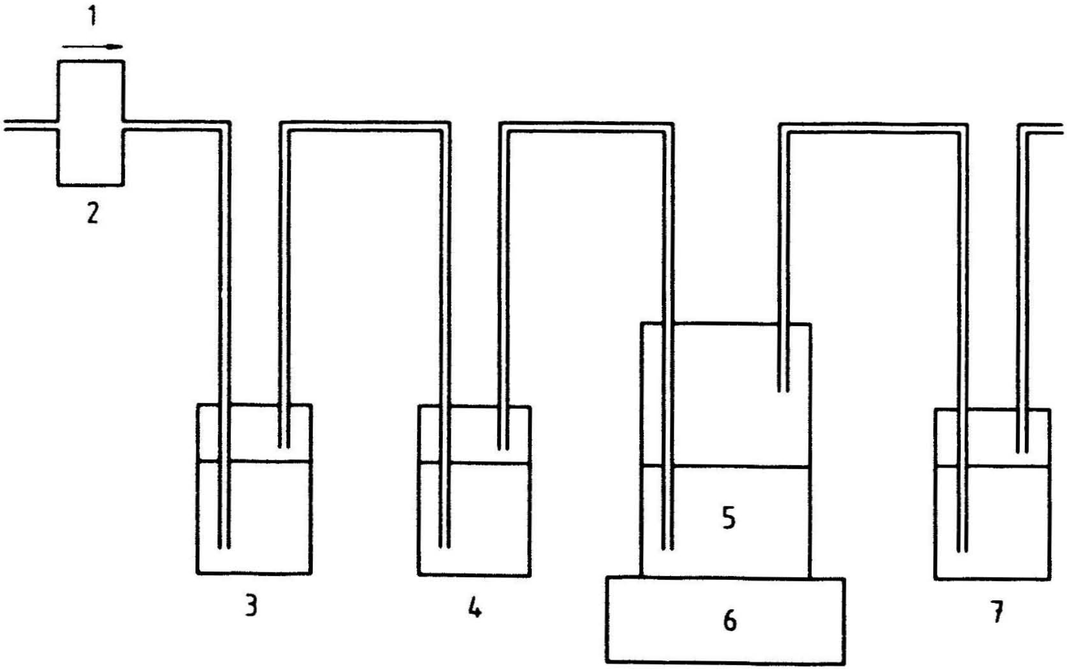
j) the colony-forming units (cfu/ml) in the inoculated test mixtures;

k) any other relevant data (e.g. initial molecular mass of the sample, molecular mass of the residual polymer).

Annex A
(informative)

Principle of a system for measuring evolved carbon dioxide (example)

The flasks are set up in series as shown in figure A.1, connected together with gas-impermeable tubing. 50 ml/min to 100 ml/min of CO_2-free air is passed through the system at a constant low pressure. Count air bubbles or use a suitable flow-rate controller to check the air-flow rate. Use synthetic CO_2-free air or compressed air. In the latter case, remove the CO_2 by passing the air through a bottle containing dry soda lime or through at least two wash bottles containing e.g. 500 ml of a 10 mol/l aqueous solution of potassium hydroxide (KOH). Use an additional flask containing 100 ml of 0,0125 mol/l barium hydroxide [$Ba(OH)_2$] solution to indicate the presence of any CO_2 in the air by turbidity. An empty flask between the indicator and the following test flask can be used to prevent liquid carry-over. CO_2 is produced in the test flask if biodegradation takes place and absorbed in the subsequent absorber bottles for determination as described in annex B.



Key

1 Compressed air
2 Flow-rate controller
3 Carbon dioxide trap (e.g. two wash bottles containing alkali)
4 Carbon dioxide indicator [$Ba(OH)_2$]
5 Test vessel
6 Stirrer
7 Carbon dioxide trap (e.g. two wash bottles containing alkali)

Figure A.1

Annex B

(informative)

Examples of methods for the determination of evolved carbon dioxide

B.1 CO_2 determination by DIC measurement

The carbon dioxide evolved is absorbed in sodium hydroxide (NaOH) solution and determined as dissolved inorganic carbon (DIC) using e.g. a DOC analyser without incineration.

Prepare a solution of 0,05 mol/l NaOH in deionized water. Measure the DIC of this solution and use this blank value when calculating the CO_2 production. Connect in series with the test flask two absorber bottles each containing 100 ml of the NaOH solution. Close the outlet of the last bottle with a small syphon to prevent CO_2 from the air from entering the NaOH solution. On the days of when the CO_2 is determined, remove the absorber bottle next to the test flask and take a sample large enough for DIC measurement (e.g. 10 ml). Replace the bottle by the second and add a new one with freshly prepared NaOH solution. On the last day, after acidification of the test solution, measure the DIC in both bottles.

Calculate the CO_2 produced using equation (B.1)

$$(CO_2)_T = \frac{(DIC_T - DIC_B) \times 3{,}67}{10} \qquad \ldots (B.1)$$

where

$(CO_2)_T$ is the mass of CO_2 evolved, in milligrams;

DIC_T is the measured DIC, in milligrams;

DIC_B is the blank DIC measured for the NaOH solution, in milligrams;

3,67 is the ratio of the molecular mass of CO_2 (44) to the atomic mass of carbon (12);

10 is a correction factor to allow for the fact that 100 ml of NaOH solution was used.

B.2 Titrimetric method using a barium hydroxide solution

The CO_2 produced reacts with the barium hydroxide [$Ba(OH)_2$] and is precipitated as barium carbonate ($BaCO_3$) [see reaction (B.2)]. The amount of CO_2 evolved is determined by titrating the remaining $Ba(OH)_2$ with hydrochloric acid (HCl) [see reaction (B.3)].

$$CO_2 + Ba(OH)_2 \rightarrow BaCO_3 + H_2O \qquad \ldots (B.2)$$

$$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O \qquad \ldots (B.3)$$

Dissolve 4,0 g of $Ba(OH)_2 \cdot 8H_2O$ in deionized or distilled water and make up to 1000 ml to obtain a 0,0125 mol/l solution. It is recommended that a sufficient amount, e.g. 5 litres, be prepared at a time when running a series of tests. Filter free of solid material and determine the exact concentration by titration with a standard HCl solution. Use phenolphthalein as indicator or an automatic titrator to determine the end-point. Store as a clear solution in a sealed flask to prevent absorption of CO_2 from the air.

Dilute 50 ml of a 1 mol/l HCl solution (36,5 g/l) to 1000 ml with deionized or distilled water to obtain a 0,05 mol/l solution.

At the start of the test, dispense exactly 100 ml of $Ba(OH)_2$ solution into each of three absorber bottles. Depending on the character and amount of the test material, use modifications of the trapping volumes. Periodically remove the bottle nearest the test vessel for titration. This should take place as needed, e.g. when the first bottle is turbid and before any precipitation of $BaCO_3$ can be observed in the second bottle. At the beginning of the test, titration may be required every other day and then every fifth day when the plateau phase is reached. After removing the absorber bottle, immediately seal it with a plug to avoid CO_2 entering from the air. Move the remaining two bottles one position closer to the test bottle and place at the end of the series a new bottle filled with fresh $Ba(OH)_2$ solution. Especially if longer test periods are used, determine the exact concentration of the solution. Handle all flasks containing test material, reference material, blank, inhibition control and inoculum control in exactly the same way.

Immediately after removing the bottle, titrate two or three aliquot portions of the $Ba(OH)_2$ solution with the HCl solution. Note the volumes of the HCl solution needed for neutralization.

Calculate the mass of CO_2 trapped in the absorber bottle is using equation (B.4):

$$m = \left(\frac{2c_B \times V_{B0}}{c_A} - V_A \times \frac{V_{Bt}}{V_{BZ}} \right) \times c_A \times 22 \qquad \text{. . . (B.4)}$$

where

m is the mass of CO_2 trapped in the absorber bottle, in milligrams;

c_A is the exact concentration of the HCl solution, in moles per litre;

c_B is the exact concentration of the $Ba(OH)_2$ solution, in moles per litre;

V_{B0} is the volume of the $Ba(OH)_2$ solution at the beginning of the test, in millilitres;

V_{Bt} is the volume of the $Ba(OH)_2$ solution at time t, before titration, in millilitres;

V_{BZ} is the volume of the aliquots of $Ba(OH)_2$ solution used for titration, in millilitres;

V_A is the volume of the HCl solution used for titration, in millilitres;

22 is half the molecular mass of CO_2.

When the following conditions apply:

- the volume of the $Ba(OH)_2$ solution before and after absorption is exactly 100 ml;
- the complete solution is used for the titration ($V_{B0} = V_{Bt} = V_{BZ}$);
- the concentration c_B of the $Ba(OH)_2$ solution is exactly 0,0125 mol/l;
- the concentration c_A of the HCl solution is exactly 0,05 mol/l;

use equation (B.5)

$$m = 1{,}1\,(50 - V_A) \qquad \text{. . . (B.5)}$$

Annex C
(informative)

Example of the determination of a carbon balance

C.1 Principle

Plastic materials are normally of more complex composition than substances with low molecular masses. The determination of CO_2 evolution or of BOD alone is often not sufficient to characterize and quantify their biodegradability. During biodegradation, new biomass is built up by the microorganisms and a part of the carbon in the test material is transformed to biomass but not biochemically oxidized. Therefore analytical parameters such as CO_2 evolution and BOD will often not reach 100 % of the respective theoretical values even in the case of complete biodegradation of a test material, and insufficient degradation could falsely be deduced from the test results. The determination of a total carbon balance, as described in this annex, may be helpful in such cases to confirm complete biodegradability. Such a balance is based on the summation of the amounts of carbon deriving from the following measurements: the carbon evolved as carbon dioxide, the carbon produced as new biomass, the carbon transformed into water-soluble organic metabolites, the carbon determined as DOC and the carbon remaining in the undegraded polymer material. The carbon sum is compared with the amount of organic carbon in the test material introduced into the test system.

C.2 Test procedure

Determine the amount of carbon dioxide evolved as described in 8.4.

Take samples of the inoculated medium at the beginning, before adding the test material, and at the end of the incubation period. Sampling must be done carefully to obtain representative samples. Pass the samples through a membrane filter or centrifuge them at about 40000 $m \cdot s^{-2}$.

Determine, for each sample, the amount of biomass on the filter or in the residue using suitable methods, e.g. by protein measurement. Determine or assume the amount of carbon in the biomass and calculate from the difference the increase in organic carbon in the biomass.

Determine, in accordance with ISO 8245, the DOC in the filtrate from each sample, and calculate the increase in organic carbon. If possible, identify the substances forming the DOC in order to confirm the production of water-soluble metabolites.

Determine the amount of carbon in the residual polymers at the end of the test using the whole of each remaining sample. This is normally a difficult procedure and can be done either directly if a polymer-specific analysis is available (see annex D) or indirectly. In the first case, extract and weigh the residual polymers and calculate from their known composition the amount of carbon. One possible method for indirect determination is to wash, dry and weigh the residue and determine the total organic carbon (TOC). Then subtract the biomass carbon (see above) from the TOC to obtain the amount of carbon in the residual polymers. Another possibility is to weigh the residue exactly and then treat it, using a suitable method, to destroy the biomass but not the polymers (this has to be checked in advance). Use e.g. sodium hypochlorite, remove the soluble part and weigh the sample again. Assume that all biomass has been removed and calculate from the mass obtained the polymer content of the residue.

C.3 Calculation of the carbon balance

Calculate the biochemically oxidized amount of carbon C_{CO_2} (mg/l) in the test material introduced into the test system (carbon content C_{MAT}) from the percentage biodegradation D_t obtained in the CO_2-evolution test (see 9.1.2), using equation (C.1):

$$C_{CO_2} = \frac{C_{MAT} \times D_t}{100} \qquad \text{. . . (C.1)}$$

Calculate the increase in biomass carbon in the test flasks containing test material C_{BIO} (mg/l) by comparing the biomass at the beginning and the end of the incubation test period, considering the measured or estimated amount of carbon in the biomass $C_{B(start)}$ and $C_{B(end)}$, as shown in equation (C.2):

$$C_{BIO} = C_{B(end)} - C_{B(start)} \qquad \text{. . . (C.2)}$$

Determine the increase in DOC during the incubation period C_{DOC} (mg/l) by comparing the DOC concentrations at the beginning $DOC_{(start)}$ and the end $DOC_{(end)}$ as shown in equation (C.3):

$$C_{DOC} = DOC_{(end)} - DOC_{(start)} \qquad \text{. . . (C.3)}$$

Determine the amount of organic carbon in the residual polymers at the end of the test C_{POL}.

Calculate the different amounts of transformed carbon as percentages of the introduced carbon C_{MAT} and sum to obtain the calculated carbon C_{CALC} (%) as shown in equation (C.4):

$$C_{CALC} = C_{CO_2} + C_{BIO} + C_{DOC} + C_{POL} \qquad \text{. . . (C.4)}$$

C.4 Example: Carbon balance of poly(β-hydroxybutyrate)[2]

Input of test material: C_{MAT} = 600 mg/l = 334,8 mg/l of carbon

Degree of biodegradation: D_t = 78 %

	$C_{B(start)}$	$C_{B(end)}$	C_{BIO}	$DOC_{(start)}$	$DOC_{(end)}$	DOC	C_{CO_2}
mg/l	3,2	61,0	57,8	2,0	22,0	20,0	261
% of C_{MAT}			17,2			6,0	78

Calculated carbon balance: C_{CALC} = 78 % + 17 % + 6 % = 101 % of C_{MAT}

---

[2] Taken from: Püchner (1994) (reference [6] in annex E).

Annex D

(informative)

Example of a determination of the amount of water-insoluble polymer remaining at the end of a biodegradation test and the molecular mass of the polymer

It may be helpful to use a procedure for measuring the amount and the molecular mass of polymers remaining at the end of a biodegradation study. The following method or another appropriate one can be used to analyse water-insoluble polymers that dissolve in organic solvents which are not miscible with water.

a) Transfer the test mixture to a funnel, add a suitable organic solvent and shake for 10 min to 20 min to extract the remaining polymers. Separate the organic solvent layer from the aqueous layer. Add fresh solvent and repeat the procedure.

b) Combine the organic extracts and evaporate the solvent until dry. Dissolve the solid sample in an appropriate volume of a suitable eluate.

c) Using a microsyringe, inject a suitable amount into a high-performance liquid chromatography (HPLC) apparatus having a column packed with a size-exclusion chromatographic gel. Start the analysis and record the chromatogram.

d) Determine the amount of polymer present using a calibration curve.

e) Determine the molecular mass of the polymer by injecting into the chromatograph the same polymer, or polymers of structure similar to that of the test polymers whose molecular masses are known. The relationship between the retention time and the molecular mass is obtained from the resulting chromatogram. Calculate the molecular mass using this relationship.

The absolute molecular mass of the test polymer can also be determined by HPLC with a combined low-angle laser-light scattering (LALLS) and differential refractive index (RI) detector.

Annex E
(informative)

Bibliography

[1] ASTM D 3536-91, *Molecular Weight Averages and Molecular Weight Distribution by Liquid Exclusion Chromatography (GEL Permeation Chromatography — GPC).*

[2] ISO 8192:1986, *Water quality — Test for inhibition of oxygen consumption by activated sludge.*

[3] ISO 11923:1997, *Water quality — Determination of suspended solids by filtration through glass-fibre filters.*

[4] MÜLLER, R.-J., AUGUSTA, J., and PANTKE, M. (1992), An Interlaboratory Investigation into Biodegradation of Plastics; Part 1: A Modified Sturm Test, *Material and Organisms*, **27**/3, pp. 179-189.

[5] PÜCHNER, P., MUELLER, W.R., and BARDTKE, D. (1995), Assessing the Biodegradation Potential of Polymers in Screening and Long-Term Test Systems, *J. Environm. Polymer Degradation*, **3**, pp. 133-143.

[6] PÜCHNER, P. (1994), Screening-Testmethoden zur Abbaubarkeit von Kunststoffen unter aeroben und anaeroben Bedingungen, Dissertation, Stuttgart University Fakultät für Bauingenieurwesen, *Stuttgarter Berichte zur Abfallwirtschaft*, **59**, Erich Schmidt Verlag, Berlin.

[7] SPERANDIO, A., and PÜCHNER, P. (1993), Bestimmung der Gesamtproteine als Biomasse-Parameter in wäßrigen Kulturen und auf Trägermaterialien aus Bio-Reaktoren, *gwf Wasser, Abwasser*, **134**, pp. 482-485.

[8] URSTADT, S., AUGUSTA, J., MÜLLER, R.-J., and DECKWER, W.-D. (1995), Calculation of Carbon Balances for the Evaluation of Biodegradability of Polymers, *J. Environm. Polymer Degradation*, **3**(3), pp. 121-131.

TECHNICAL CORRIGENDUM 1

Technical Corrigendum 1 to ISO 14852:1999 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

Page 7, Subclause 8.3

In line 2 of the Note following the second paragraph, replace "10^{-6}" by "10^3 to 10^6".

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of the these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard alongwith amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

This Indian Standard has been developed from Doc: No. PCD 12 (2390).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters :

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002
Telephones : 2323 0131, 2323 3375, 2323 9402 *Website* : www.bis.org.in

Regional Offices :

		Telephones
Central	Manak Bhavan, 9 Bahadur Shah Zafar Marg NEW DELHI 110 002	2323 7617 2323 3841
Eastern	1/14 C.I.T. Scheme VII M, V.I.P. Road, Kankurgachi KOLKATA 700 054	2337 8499, 2337 8561 2337 8626, 2337 9120
Northern	SCO 335-336, Sector 34-A, CHANDIGARH 160 022	260 3843 260 9285
Southern	C.I.T. Campus, IV Cross Road, CHENNAI 600 113	2254 1216, 2254 1442 2254 2519, 2254 2315
Western	Manakalaya, E9 MIDC, Marol, Andheri (East) MUMBAI 400 093	2832 9295, 2832 7858 2832 7891, 2832 7892

Branches : AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. PARWANOO. PATNA. PUNE. RAJKOT. THIRUVANANTHAPURAM. VISAKHAPATNAM.

Printed by the Manager, Govt. of India Press, Faridabad